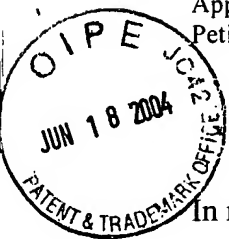


JFW/1615

Appln. No.: 10/009,583
Petition to Director Under 37 CFR 1.181

Docket No. 8830-10 (157952)



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Patent application of
Louise Georgina Buttle
Serial No.: 10/009,583
Filed: March 19, 2002
For: PIGMENT
Group Art Unit:
1615
Examiner:
Micah Paul Young
Conf. No: 1753

Petition to Director Under 37 C.F.R. 1.181

Mail Stop Petitions
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450.

Sir:

This is a Petition to the Director Under 37 CFR 1.181 and is in response to the Advisory Action mailed on April 5, 2004 in the above referenced matter. The Advisory Action was mailed after the expiration of the six month period for responding to the office action. Thus, the application is technically abandoned. However, as set forth below, the entry of the prior response was timely and overcame the pending rejections. Thus the application should not have been abandoned. The Office of Petitions recommended filing this Rule 1.181 Petition and stated that no fee should be due. However, if any fee is determined to be due, kindly charge such fee to deposit account 50-0573.

CERTIFICATE OF MAILING UNDER 37 C.F.R. 1.8(a)	
I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date indicated below, with sufficient postage, as first class mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.	
BY	<i>James M. McHenry</i>
DATE:	<i>June 16, 2004</i>

Statement of Facts

1. A Final Office Action was mailed on September 24, 2003, rejecting claims 1, 3, 4, 8 and 10-14 under 35 U.S.C. 103(a) as being unpatentable over XP 000939130. The Office Action contained no enablement rejection. [Exhibit 1]

2. A Response to the Office Action was mailed on December 22, 2003, providing arguments traversing the rejection of the claims and including amendments to the claims. [Exhibit 2]

3. On February 12, 2004, a first Advisory Action was mailed from the United States Patent and Trademark Office. The Advisory Action stated that the Response would not be entered. The basis for the rejection was stated as follows:

“The examiner agrees with applicant that salmonid and carp fish have different processes for metabolizing carotenoids. The claims however are drawn to pigments in general. Applicant has not shown that all pigments are processed [sic] differently between the two species.”

Thus, the Examiner was in agreement that applicant's arguments overcame the rejection but required that the claims be brought into conformance with the arguments. [Exhibit 3]

4. Applicant's representative contacted Examiner Young on March 2, 2004 to discuss amending the claims to limit them to carotenoid pigments. The Examiner agreed that amending claim 1 to limit the claims to carotenoid pigment would render the claims allowable. [Summarized in Exhibit 4]

5. A Response Under 37 CFR 1.116 was filed on March 11, 2004, with the appropriate extension fee. The response amended the claims to limit the pigment to a carotenoid pigment as the Examiner had agreed. [Exhibit 4]

6. A second Advisory Action was mailed on April 5, 2004. The Advisory Action indicated that the Response that was filed on March 11, 2004 was entered. The Advisory Action however, continued to reject the claims, However the rejection was based on different grounds:

“Upon further review of the specification, there does not seem to be a patentable distinction made between those feeds containing cholesterol and those not containing chloesterol [sic]. The effect of the cholesterol is not fully explained or established in the specification of [sic] in the claims. The examples cite statistical data, yet the data points have SD overlaps which on face value bring into question the importance of the cholesterol. The importance of the cholesterol is the uptake enhancement of the pigment must be more clearly pointyed [sic] out and claimed

in order for the prosecution to continue further.
[Exhibit 5]

7. It is clear from this explanation that the examiner had raised a new ground for rejecting the claims. Furthermore, since there was no further rejection based on the art, it is clear that the amendments overcame the examiner's prior rejection. It is also clear that the amendments played no part in the new rejection of the claims. Thus, the examiner's statement for rejecting the claims should have been issued as part of a new office action, not in an Advisory Action mailed after the statutory period expired.

8. The facts establish that, upon entry of the March 11, 2004 Response, all the claim rejections had been overcome. Applicant does not dispute that, if that facts justify, an examiner may issue a new rejection after a prior rejection is overcome. However, the rules require that the rejection be part of a new office action. Once a rejection is overcome, the examiner has only two choices: issue a new action or a Notice of Allowance. The examiner did neither.

9. In the present case the examiner foreclosed any chance for the Applicant to respond to the rejection by issuing an Advisory Action after the expiration of the statutory period. Simply put, the examiner's action was improper. The examiner should have issued a new office action, with a new statutory period for responding to the new rejection. Furthermore, since the amendments that were made to the claims had no bearing on the examiner's issuance of a new rejection, any new action would be non-final.

10. Based on the foregoing, Applicant respectfully requests that the Director exercise its authority and withdraw the abandonment of the application, and direct the examiner to issue a new non-final action on the merits or a Notice of Allowance.

Respectfully submitted,

LOUISE GEORGINA BUTTLE



ROBERT E. CANNUSCIO
Registration No. 36,469
DRINKER BIDDLE & REATH, LLP.
One Logan Square
18th and Cherry Streets
Philadelphia, PA 19103-6996
(215) 988-3312 - Telephone
(215) 988-2757 - Fax
Attorney for Applicant



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/009,583	03/19/2002	Louise Georgina Buttle	8830-10 (157952)	1753

23973 7590 09/24/2003

DRINKER BIDDLE & REATH
ONE LOGAN SQUARE
18TH AND CHERRY STREETS
PHILADELPHIA, PA 19103-6996

EXAMINER

YOUNG, MICAH PAUL

ART UNIT

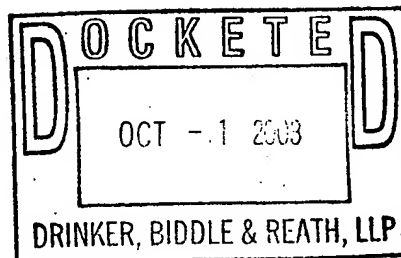
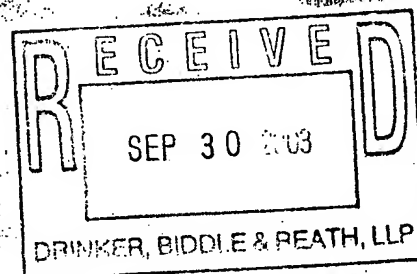
PAPER NUMBER

1615

DATE MAILED: 09/24/2003

10

Please find below and/or attached an Office communication concerning this application or proceeding.



Office Action Summary	Application No.	Applicant(s)	
	10/009,583	BUTTLE, LOUISE GEORGINA	
	Examiner	Art Unit	
	Micah-Paul Young	1615	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

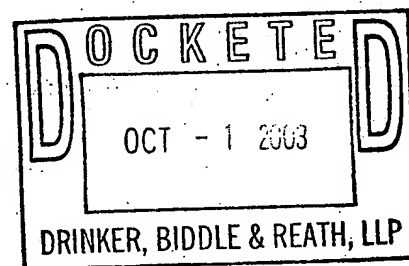
- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 30 June 2003.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1,3,4,8 and 10-14 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1,3,4,8 and 10-14 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.



Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
 Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- 11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.
 If approved, corrected drawings are required in reply to this Office action.
- 12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

- 13) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 a) ☐ All b) ☐ Some * c) ☐ None of:
 1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
 * See the attached detailed Office action for a list of the certified copies not received.
- 14) ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).
 a) ☐ The translation of the foreign language provisional application has been received.
- 15) ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) Paper No(s). _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ | 6) <input type="checkbox"/> Other: |

DETAILED ACTION

Acknowledgment of Papers Received: Amendment B dated 6/30/03.

Claim Rejections - 35 USC § 103

1. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

2. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.
3. Claims 1, 3, 4, 8, 10-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Iwahashi et al (XP 000939130). The claims are drawn to a method of feeding fish cholesterol in order to improve their pigment.

The reference teaches methods for improving the pigment of fancy carp, by including cholesterol and a pigment into their feed compositions (Abstract). The carp were split into 10 separate groups with each given different amounts of various combinations of pigment and cholesterol. Group 8 was given a combination of astaxanthin and cholesterol (Table I). The researchers observed an increase in the intensity of the redness of the fancy carp after the feeding (Table 4). In Group 8 the accumulation rate of carotenoids was 1.41 % (Table 7).

What is lacking in the reference is physical form of the feed composition. This however would be obvious to a skilled artisan since most feed compositions are presented in pellet, of tablet form. Also the concentration of cholesterol is slightly higher than that of applicant. Though the reference does not disclose the specific concentrations of the claimed invention,

Art Unit: 1615

applicant is reminded that it is well within the level of ordinary skill in the art to find the optimal working ranges for a composition. Where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation. *See In re Aller*, 220 F.2d 454 105 USPQ 233, 235 (CCPA 1955).

Furthermore the claims differ from the reference by reciting various concentrations of the active ingredients. However, the preparation of various feed compositions having various amounts of the active is within the level of skill of one having ordinary skill in the art at the time of the invention. It has also been held that the mere selection of proportions and ranges is not patentable absent a showing of criticality. *See In re Russell*, 439 F.2d 1228 169 USPQ 426 (CCPA 1971).

With this in mind a skilled artisan would have followed the suggestions and teachings of the art. A skilled artisan would have been motivated by the teachings of Iwahashi to optimize the concentrations of pigment and cholesterol, in order to improve the flesh color of fancy carp, or any fish benefiting from increased pigmentation. These fish are more appealing to consumers, and are easier to market to consumers. It would have been obvious to one of ordinary skill in the art, at the time of the invention to follow these teachings and suggestions with an expected result of a method to improve pigmentation and feed composition to do so.

Response to Arguments

4. Applicant's arguments filed 6/30/03 have been fully considered but they are not persuasive. Applicant argues that:

- a. The reference does not improve the color of the flesh of the fish and nearly improves the color of the skin.

Art Unit: 1615

Applicant is reminded that the claims are drawn to a method comprising feeding fish cholesterol, which is taught by the reference. A reference does not need to include all of the elements of the claimed invention in order to obviate it. The fish in the reference are fed cholesterol, which is the essential element of the claimed invention. Applicant is invited to provide evidence to the difference in the procedures, which lead to the distinctiveness of the claimed invention. It is the position of the examiner that since Iwahashi discloses the essential elements of feeding fish a diet of cholesterol to improve their pigment, the reference will continue to obviate the instant claims.

Conclusion

5. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire **THREE MONTHS** from the mailing date of this action. In the event a first reply is filed within **TWO MONTHS** of the mailing date of this final action and the advisory action is not mailed until after the end of the **THREE-MONTH** shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than **SIX MONTHS** from the mailing date of this final action.

Application/Control Number: 10/009,583

Page 5

Art Unit: 1615

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Micah-Paul Young whose telephone number is 703-308-7005.

The examiner can normally be reached on M-F 7:00 am - 3:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Thurman K Page can be reached on 703-308-2927. The fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-1234.

Micah-Paul Young
Examiner
Art Unit 1615

571-272-0608

MP Young


THURMAN K. PAGE
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

Commissioner of Patents and
Trademarks

Please acknowledge receipt of the attached (specified below)
by date stamping and returning this pre-addressed postcard.

File # 8830-10 (157952)

Serial/Patent/Registration # 10/009,583

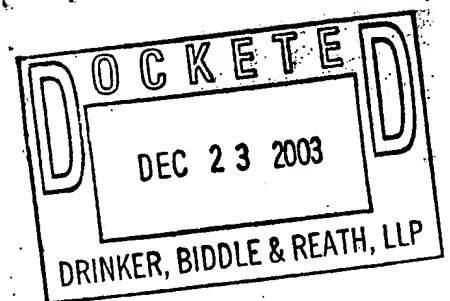
Matter: PIGMENT

- ☐ Application w/Drawing
- ☐ Specimens (_____)
- ☐ Trans. Ltr. w/_____Copies
- ☒ Response to Final O.A.
- ☐ Communication
- ☐ A.A.U./S.O.U./E.O.T.S.O.U.
- ☐ Decl. Under Sec. _____
- ☐ Renewal Application
- ☐ Ext. Time Oppose/Not. Opp.
- ☐ Specification (_____pgs.)
- ☐ Claims (_____pgs.)

- ☐ Abstract (_____pgs.)
- ☐ Drawings (_____sheets)
- ☐ Decl. and P.O.A.
- ☐ Priority Document
- ☐ Amendment
- ☐ Assignment/Merger/Chg. Nm.
- ☐ Other _____

- ☐ Final Fee
- ☐ Fee \$ n/a
(Dep. Act. 50-0573)

EXP MAIL # Regular Mail - December 22, 2003



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Patent application of
Louise Georgina Buttle
Serial No.: 10/009,583
Filed: March 19, 2002
For: PIGMENT

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: Group Art Unit:
: 1615
:
: Examiner:
: Micah Paul Young
:
: Conf. No: 1753

Response Under 37 C.F.R. 1.116

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450.

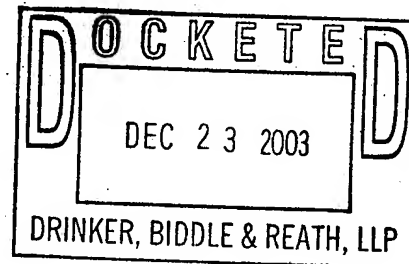
**RESPONSE UNDER 37 CFR 1.116
- EXPEDITED PROCEDURE -
EXAMINING GROUP ART UNIT**

Sir:

This is in response to the office action mailed September 24, 2003 (paper no. 10). This paper is being submitted within the three month shortened statutory period for reply, and thus no fee is believed due. If any fees are required, please charge Deposit Account No. 50-0573.

Amendments to the Claims begin on pg. 2 of this paper

Remarks/Argument begin on pg. 5 of this paper.



**CERTIFICATE OF MAILING
UNDER 37 C.F.R. 1.8(a)**

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date indicated below, with sufficient postage, as first class mail, in an envelope addressed to: MS AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

BY: *James M. McNeely*

DATE: *December 22, 2003*

Amendments to the Claims:

The following listing of claims replaces all previous claim listings.

1. (Currently Amended) A method of enhancing the uptake of pigment by fish of a salmonid species to induce a change in the pigmentation of the flesh, said method comprising the step of feeding fish with a feed having a total pellet weight, wherein the feed comprises pigment and cholesterol, and wherein the cholesterol is added to the range of 0.1-5% of the total pellet weight.

2. (Previously Cancelled)

3. (Previously Amended) A method as claimed in claim 1 wherein cholesterol comprises between 1-4% of the total pellet weight.

4. (Previously Amended) A method as claimed in claim 1 wherein cholesterol comprises between 1-3% of the total pellet weight.

5-7. (Previously Cancelled)

8. (Cancelled)

9. (Previously Cancelled)

10-11 (Cancelled)

12. (Currently Amended) The method of claim 8 1, wherein the salmonid species is Atlantic salmon, Coho salmon, Chinook salmon, Rainbow trout, or Arctic charr.

13-14 (Cancelled)

Remarks/Argument

Claims 1, 3, 4, 8 and 10-14 are pending in the application. Claims 8, 10-11 and 13-14 have been cancelled without prejudice. Claims 1 and 12 have been amended. After entry of this amendment, claims 1, 3, 4 and 12 will be pending.

The amendments to the claims place the claims in better form for allowance, as discussed below. Entry of the present response is therefore proper, and reconsideration of the claims is respectfully requested based on the above changes and the remarks set forth below.

PTO 1449 Forms

Applicant acknowledges that the Examiner has initialed and returned the PTO 1449 form submitted with the IDS on November 6, 2001. Applicant respectfully requests that the Examiner initial the PTO Form 1449 submitted with the Supplemental IDS filed on March 12, 2002, and return a copy to Applicant's undersigned representative.

Response to the section 103(a) rejection

Claims 1, 3, 4, 8 and 10-14 are rejected under 35 U.S.C. 103(a) as allegedly rendered obvious by Iwahashi et al., Bulletin of the Japanese Society of Scientific Fisheries, Vol. 42, No. 12, Pages 1339-1344 (1976), hereinafter "Iwahashi." Claims 8, 10-11 and 13-14 have been cancelled without prejudice, and the rejection is moot as to these claims. Claims 1, 3, 4 and 12, as amended, are non-obvious over Iwahashi for the reasons discussed below.

In an earnest attempt to advance prosecution, claim 1 has been amended to recite a method of enhancing the uptake of pigment in *salmonid* fish, by feeding the salmonid fish with feed comprising pigment and cholesterol. Specific salmonid species are identified in claim 12 as amended.

Iwahashi discloses that skin pigmentation in decorative *carp* can be improved by the addition of carotenoids into the diet of the fish. Although some of the feeds discussed in Iwahashi contain cholesterol, this cholesterol had no effect on the accumulation of carotenoids in the skin of the decorative *carp* (see Iwahashi abstract). Iwahashi also does not disclose or suggest that test feeds which contain cholesterol can influence flesh color of a *salmonid* fish.

The accumulation of pigment in various tissues of fish is influenced by the metabolic pathway by which the pigment is processed. Different fish species process and store pigment differently. For example, salmonid fish have a "reductive" pathway for metabolizing carotenoids, while carp species have an "oxidative" pathway for metabolizing carotenoids. See Bjerking et al., Comparative Biochemistry and Physiology Part B (2000) 125: 395-404 (copy enclosed), at pg. 402. One skilled in the art would therefore not expect that processing and deposition of pigment in carp tissues would indicate how pigment is processed and deposited in tissues of salmonid species. Iwahashi therefore does not provide one skilled in the art with the motivation to feed cholesterol-containing feed to salmonid fish for enhancing flesh color. Iwahashi also does not provide one skilled in the art with any reasonable expectation that the color of salmonid flesh could be successfully enhanced with cholesterol-containing feed. Applicants therefore respectfully request that the 35 U.S.C. 103(a) rejection of claims 1, 3, 4 and 12 be withdrawn.

Conclusion

The claims of the application are believed to be in condition for allowance. An early action toward that end is earnestly solicited.

Respectfully submitted,

LOUISE GEORGINA BUTTLE



ROBERT E. CANNUSCIO

Registration No. 36,469

DRINKER BIDDLE & REATH, LLP.

One Logan Square

18th and Cherry Streets

Philadelphia, PA 19103-6996

(215) 988-3312 - Telephone

(215) 988-2757 - Fax

Attorney for Applicant



Comparative Biochemistry and Physiology Part B 125 (2000) 393–404

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Astaxanthin and its metabolites idoxanthin and crustaxanthin in flesh, skin, and gonads of sexually immature and maturing Arctic charr (*Salvelinus alpinus* (L.))

B. Bjerkeng^{a,*}, B. Hatlen^{b,1}, M. Jobling^b^a Akvaforsk, Institute of Aquaculture Research AS, N-6600 Sundalsøra, Norway^b The Norwegian College of Fishery Science, University of Tromsø, N-9007 Tromsø, Norway

Received 16 September 1999; received in revised form 3 December 1999; accepted 6 December 1999

Abstract

Carotenoid compositions of the flesh, skin, and ovaries were determined in sexually maturing and immature Arctic charr (*Salvelinus alpinus*) fed diets supplemented with astaxanthin (optical isomer ratio (3*S*,3'*S*): (3*R*,3'*S*; *meso*): (3*R*,3'*R*): 1:2:1). Astaxanthin comprised 64–79% of the flesh carotenoids, and the 3',4'-*cis* and 3',4'-*trans* glycolic isomers of idoxanthin, present in a 1:1 ratio, represented 20–35%. The flesh of the sexually maturing charr contained relatively more idoxanthin than that of sexually immature fish (20 vs 35% of total carotenoids), possibly being indicative of a higher metabolic turnover of astaxanthin in the latter. The relative proportions of flesh carotenoids were unaffected by sex. The relative carotenoid composition of ovaries was similar in sexually maturing and immature females. The 3',4'-*cis* and 3',4'-*trans* glycolic isomers of idoxanthin (ratio 0.7:1) were the major carotenoids (56% of total), followed by crustaxanthin (20%), and astaxanthin comprised less than 5% of ovarian carotenoids. Three glycolic isomers of crustaxanthin were detected (3,4,3',4'-*di-cis*-3,4'-*cis*-3',4'-*trans*-3,4,3',4'-*di-trans*-glycolic isomer ratio 2.6:3.1:1) in the ovaries. Sex and maturity status had no apparent effect on the relative composition of skin carotenoids. The skin carotenoids consisted mainly of diesters (82–87% of total carotenoids) and monoesters (7–13% of total carotenoids). Saponification revealed that astaxanthin comprised 85% and idoxanthin 10% of total carotenoids, and minor amounts of ruinoxanthin-, lutein-, and zeaxanthin-like metabolites were also present. Maturity status seems to be more important than sex in determining the relative carotenoid composition of the tissues of Arctic charr, with astaxanthin and its metabolites being selectively accumulated in different tissues. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Astaxanthin; Idoxanthin; Crustaxanthin; Metabolism; Sexual maturation; Arctic charr; *Salvelinus alpinus*

1. Introduction

Maturing salmonid fishes redistribute their body pool of carotenoid pigments, there being a transfer of flesh carotenoids to the skin and gonads (Steven, 1949; Crozier, 1970; Ando and Hatano, 1987; Torrissen and Naevdal, 1988; Bjerkeng et al., 1992; Synowicki et al., 1993; Hatlen et al., 1995, 1996). This redistribution results in a reduction in flesh carotenoid concen-

* Corresponding author. Tel.: +47-71695305; fax: +47-71695301.

E-mail address: bjorn.bjerkeng@akvaforsk.nlh.no (B. Bjerkeng).

¹ Present address: NorAqua Innovation AS, P.O. Box 23, N-4335 Dirdal, Norway.

tration and may also be associated with a loss of whole-body carotenoids (Crozier, 1970; Bjerkeng et al., 1992). Recent evidence suggests that carotenoid redistribution in Arctic charr is influenced by the steroid sex hormones 11-ketotestosterone and 17 β -estradiol (Bjerkeng et al., 1999).

The major carotenoids of the flesh of cultured Arctic charr are astaxanthin (3,4'-dihydroxy- β , β -carotene-4,4'-one) and idoxanthin (3,3',4'-trihydroxy- β , β -carotene-4-one), a reductive metabolite of dietary astaxanthin (Aas et al., 1997; Hatlen et al., 1997). The proportion of idoxanthin tends to decrease as the fish increase in size (Aas et al., 1997), but little is known about any changes in carotenoid composition that may accompany sexual maturation in the Arctic charr.

Most studies on carotenoid compositions of skin and ovaries of *Salvelinus* species have been performed on fish captured from the wild (Scalia et al., 1989a; Ando et al., 1990a, 1991), but under such circumstances it is not possible to distinguish between carotenoids accumulated directly from the diet and those derived from astaxanthin metabolism. In the present experiment, Arctic charr were fed astaxanthin as the only dietary carotenoid supplement, and the qualitative carotenoid compositions of flesh, skin and ovaries of sexually immature and mature Arctic charr studied, with emphasis on the astaxanthin metabolites idoxanthin and crustaxanthin (3,4,3',4'-tetrahydroxy- β , β -carotene) were investigated.

2. Materials and methods

2.1. Biological material and carotenoid extracts

Arctic charr of the Hammerfest strain were used in the experiment, and details regarding rearing conditions, growth, and flesh carotenoid concentrations were reported by Hatlen et al. (1996). The fish had an average initial weight of 350 ± 63 g (mean \pm SD), and average final weights were 1334 ± 179 , 1271 ± 39 , 979 ± 72 and 763 ± 62 g for immature male, immature female, mature male and mature female fish, respectively. The fish were held in fresh water (ambient temperature maximum 15.3°C end of July, minimum 1°C beginning of November) with over 60% oxygen saturation, under a simulated natural photoperiod (70°N). The charr were fed dry feed pellets (T.

Skretting AS, Stavanger, Norway) declared to contain 50 and 80 mg astaxanthin kg⁻¹, during weeks 0–12 and 13–27, respectively. Feed astaxanthin contents, as analysed by isocratic HPLC (Schüep and Schierle, 1995), were 49 and 74 mg kg⁻¹, respectively. The astaxanthin source (Carophyll Pink, Hoffmann-La Roche, Basel, Switzerland) consisted of a 1:2:1 mixture of the (3S,3'S)-, (3R,3'S; meso)- and (3R,3'R) optical isomers of astaxanthin, and 85% all-E-astaxanthin and 15% Z-astaxanthins.

The first ripe males were observed on 26th September, ovulating females on 27th October, and fish were sampled on 3rd November. The fish were killed by a blow to the head, weighed and gutted. Maturation status was assessed by examination of gonads. Skin, flesh and gonads were sampled, and pooled for immature male ($n=6$), immature female ($n=5$), mature male ($n=10$) and mature female fish ($n=11$), respectively. General precautions for treatment of carotenoids were taken during storage and handling of carotenoid samples (Schiedt and Liaaen-Jensen, 1995). Samples were protected from direct sunlight, and stored at -80°C in darkness until analyzed. Carotenoids from samples of flesh, skin and ovaries were extracted repeatedly with acetone-methanol (7:3), and the extracts were then evaporated, and re-dissolved in 20% acetone in *n*-hexane (1 ml). The resulting solutions were filtered (0.45 μm ; Minisart SRP15, Sartorius, Germany) directly into the sample vials, which were immediately sealed. The samples were analyzed by high pressure liquid chromatography (HPLC) the same day.

2.2. HPLC conditions

Two isocratic HPLC systems were used. System I consisted of a Spherisorb SS-CN nitrile column (PhaseSep, Queensferry, Clywd, UK; length 250 mm; internal diameter 4.6 mm; particle size 5 μm), using 20% acetone in hexane as the mobile phase (flow rate 1.5 ml min⁻¹; pressure approximately 52 bar). System II consisted of a H₂PO₄-modified silica gel column (Hibar, LiChrosorb SI 60, 5 μm particles; internal diameter, 4.6 mm, length 125 mm; Merck, Darmstadt, Germany) as described by Vecchi et al. (1987). The mobile phase was 20% acetone in hexane, and the flow rate was 1.2 ml min⁻¹ (pressure approximately 36 bar). The mobile phases were renewed each day. The Shi-

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madzu LC-10AS Liquid chromatograph was connected to a Shimadzu SPD-M6A Photodiode array UV-Vis detector and Shimadzu CBM-10A Communications bus module, and the detection wavelength was set to 470 nm. Sample application was performed by a Shimadzu SIL-10 auto injector. All chromatograms were reintegrated (Class LC10 software, Shimadzu, Japan) for baseline adjustment.

2.3. Hydrolysis of carotenol esters

Carotenoids from the skin samples were saponified according to Bjerkeng et al. (1990), using methanolic KOH (0.18 mol l⁻¹) in CH₂Cl₂. Oxygen was removed by flushing with nitrogen to reduce formation of autoxidation products of α -ketols (Kuhn and Sørensen, 1938).

2.4. Preparation of reference compounds

Authentic standards of the 3',4'-*cis* and 3',4'-*trans* glycolic isomers of idoxanthin were prepared from chemically synthesized all-*E*-astaxanthin (Carophyll Pink, Hoffmann-La Roche, Basel, Switzerland), having a 1:2:1 ratio of the (3*R*,3'*R*)-, (3*R*,3'*S*)- and (3*S*,3'*S*)-isomers. Standards were prepared by reduction with NaBH₄ in dry diethyl ether for 10 min, as described by Aas et al. (1997). Authentic standards of the 3,4,3',4'-*di-cis*, 3,4-*cis*-3',4'-*trans*, and 3,4,3',4'-*di-trans* glycolic isomers of crustaxanthin were prepared similarly, by prolonging the reaction time (1 h). The reaction was monitored by thin layer chromatography (TLC) on silica gel plates (0.2 mm; Kieselgel 60 G, Product no. 7731; Merck, Darmstadt, Germany) using 50% acetone in hexane as the mobile phase. The reaction was terminated by addition of water saturated with NaCl, and the carotenoids were then extracted with diethyl ether. The relative yields were 32.4 and 67.6% for the 3',4'-*cis* and 3',4'-*trans* glycolic isomers of idoxanthin, and 8.7, 41.8 and 49.5% for the 3,4,3',4'-*di-cis*, 3,4-*cis*-3',4'-*trans*, and 3,4,3',4'-*di-trans* glycolic isomers of crustaxanthin, respectively. Reference compounds were stored refrigerated (-80°C) under nitrogen gas until used.

2.5. Determination of individual carotenoids

Individual carotenoids were quantified from HPLC chromatogram areas, and corrected for

differences in molar absorptivities ($E_{1\%}^{1\text{cm}}$) at the detection wavelength (470 nm). The $E_{1\%}^{1\text{cm}}$ -values used were 2100 for all-*E*-astaxanthin (Britton, 1995), and 1350 and 1750 for 13*Z*- and 9*Z*-astaxanthin, respectively. The $E_{1\%}^{1\text{cm}}$ -values for 13*Z*- and 9*Z*-astaxanthin were estimated based on the HPLC response factors relative to all-*E*-astaxanthin reported by Schöep and Schierle (1995). An $E_{1\%}^{1\text{cm}}$ -value of 2245 (acetone, $\lambda_{\text{max}} = 458$ nm) was used for the 3',4'-*cis* and 3',4'-*trans* glycolic isomers of idoxanthin (K. Schiedt, personal communication), and an $E_{1\%}^{1\text{cm}}$ -value of 2800 (acetone, $\lambda_{\text{max}} = 452$ nm) for the 3,4,3',4'-*di-cis*, 3,4-*cis*-3',4'-*trans*, and 3,4,3',4'-*di-trans* glycolic isomers of crustaxanthin. Astaxanthin was used as an external standard for quantification of individual carotenoids. Standards of known concentration were prepared from crystalline all-*E*-astaxanthin (Hoffmann-La Roche Ltd, Basel, Switzerland) and these were run, each time samples were analysed. The concentration of the standard solution was measured spectrophotometrically (UV-260, Shimadzu, Japan) using $E_{1\%}^{1\text{cm}} = 2100$ at absorbance maximum ($\lambda_{\text{max}} = 472$ nm). Spectral fine structures for VIS-spectra are expressed as %III/II (Ks et al., 1970). Individual carotenoids (astaxanthin, idoxanthin, and crustaxanthin) were characterized by HPLC and TLC, co-chromatography tests, and on-line VIS-spectra. Using HPLC-system I, the retention times (t_r) were 10.8, 14.4 and 19.0 min for the 3,4,3',4'-*di-cis*, 3,4-*cis*-3',4'-*trans*, and 3,4,3',4'-*di-trans* glycolic isomers of crustaxanthin, respectively. All crustaxanthin isomers exhibited the following VIS-spectra absorbance bands (20% acetone in *n*-hexane) (420), 452 and 479 nm, and the spectral fine structure, %III/II, was 26–30.

3. Results and discussion

The results indicate that dietary astaxanthin is extensively metabolized by the reductive pathway in Arctic charr, and the general findings are in good agreement with those obtained for other *Salvelinus* species (Ando et al., 1990a,b, 1991; Ando and Hatano, 1991).

3.1. Flash carotenoids

In flesh, idoxanthin comprised 20–35% of total carotenoids (Table 1), and the ratio of the 3',4'-*cis*

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and 3',4'-*trans* glycolic isomers was ca. 1:1. The relative concentration of idoxanthin found here was lower than previously reported for Arctic charr (46-76% of total carotenoids; Aas et al., 1997; Hatten et al., 1997; Bjerkeng et al., 1999). Crustaxanthins were not detected, and only small amounts of crustaxanthins have been detected in the flesh of Arctic charr in previous studies (Aas et al., 1997). Similarly, a crustaxanthin concentration of less than 5% of total carotenoid was found in the *Salvelinus* species investigated by Ando et al. (1990a,b, 1991) and Ando and Hatano (1991). There are indications that astaxanthin and canthaxanthin may be deposited unchanged in the flesh of cultured Arctic charr (Christiansen and Wallace, 1988; Boyer and Van Toever, 1993; Shahidi et al., 1993, 1994; Synowiecki et al., 1993), but astaxanthin metabolites may have escaped detection by the analytical procedures used in some previous studies. The 9Z-, 13Z-, and 15Z geometrical stereoisomers of astaxanthin comprised less than 5% of total astaxanthin of the flesh of the charr examined in the current study, a considerably lower proportion than the Z-isomers of astaxanthin present in the diet (ca. 20%). This is in agreement with recent findings for rainbow trout (*Oncorhynchus mykiss*), which indicate that absorption of astaxanthin Z-isomers is considerably less than that of all-E-astaxanthin (Bjerkeng et al., 1997; Østerlie et al., 1999).

Maturing fish had a higher relative idoxanthin, and lower relative astaxanthin concentration, in the flesh than sexually immature fish (Table 1). In both Atlantic salmon (*Salmo salar*; Schiedt, 1989; Schiedt et al., 1989) and Arctic charr (Aas et al., 1997) the relative proportion of idoxanthin tends to decrease as fish increase in age or size, but the present results indicate that this trend may be

reversed during sexual maturation. The relative carotenoid composition was similar in male and female fish, but results of another recent study suggest that there may be differences in flesh carotenoid composition between the sexes of Arctic charr: the flesh of females seems to contain relatively more idoxanthin than that of the males (Bjerkeng et al., 1999).

3.2. Ovarian carotenoids

HPLC-system 1 was well suited for the separation of the 3,4(3',4')-*cis* and *trans* glycolic isomers of idoxanthin and crustaxanthin, but was less suitable for separation of carotenols with a polarity similar to or less than that of astaxanthin (Fig. 1). Astaxanthin comprised less than 5% of the total carotenoids of the ovaries, and, in HPLC-system 1, astaxanthin co-eluted with an unidentified yellow xanthophyll. Crustaxanthins comprised ca. 20%, and idoxanthins ca. 56% of the ovarian carotenoids in both immature and maturing charr (Table 2). The ratio of 3',4'-*cis* to 3',4'-*trans* glycolic isomer of idoxanthin was approximately 0.7:1. The relative concentration of crustaxanthin was within the range reported for other *Salvelinus* species (Ando et al., 1990a,b, 1991; Ando and Hatano, 1991). Only minor amounts of carotenoid tetrals have been detected in the ovaries of species other than salmonids (Miki et al., 1982; Matsuno et al., 1985).

Crustaxanthin has four asymmetric carbons, and there are ten different optical R/S isomers. A random reduction of any optical isomer of astaxanthin to crustaxanthin would lead to a 1:2:1 distribution between the 3,4,3',4'-di-*cis*-, 3,4-*cis*-3',4'-*trans*-, and 3,4,3',4'-di-*trans*-glycolic isomers of crustaxanthin. The chemical reduction of astax-

Table 1

Relative carotenoid compositions of the flesh of sexually immature and maturing male and female Arctic charr

	Immature		Maturing	
	Male	Female	Male	Female
Astaxanthin, % of total carotenoids	78	79	64	64
Iodoxanthin, % of total carotenoids	21	20	35	35
3',4'- <i>Cis</i> , % of idoxanthin	53	53	50	51
3',4'- <i>Trans</i> , % of idoxanthin	48	47	50	49
Crustaxanthin	ND ^a	ND	ND	ND
Other carotenoids, % of total	1	1	1	1

^a ND = not detected.

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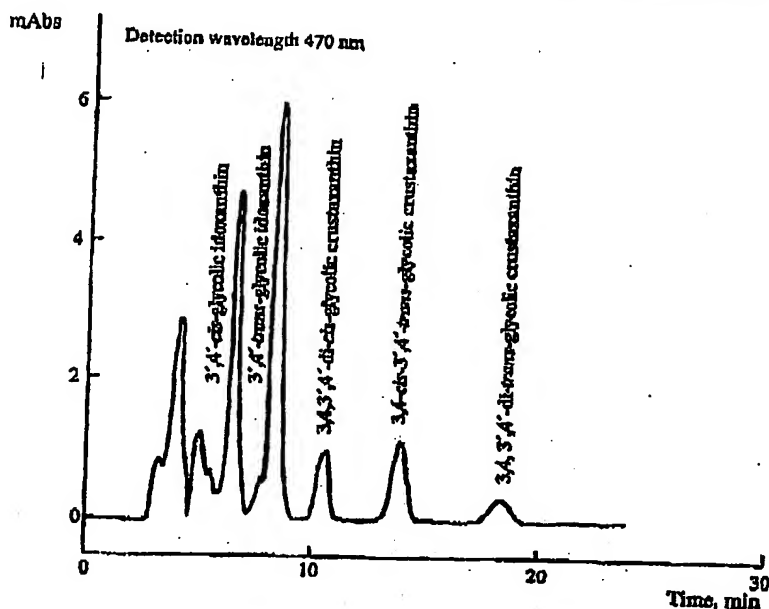


Fig. 1. Separation of 3',4'-*cis* and 3',4'-*trans* glycolic isomers of idoxanthin, and the 3,4,3',4'-*di-cis*-, 3,4-*cis*-3',4'-*trans*-, and 3,4,3',4'-*di-trans*-glycolic isomers of crustaxanthin, extracted from ovaries of Arctic charr. Separation was by normal phase HPLC on a nitrile column, using acetone-*n*-hexane (20:80) as the mobile phase, and the flow was 1.5 ml min⁻¹.

anthin to crustaxanthin with NaBH₄, showed that the favoured product was the *trans*-glycolic form, the chemical reduction resulting in a mixture of 3,4,3',4'-*di-cis*-, 3,4-*cis*-3',4'-*trans*-, and 3,4,3',4'-*di-trans*-glycolic crustaxanthin isomers with a ratio of 0.18:0.84:1. We separated and quantified the diastereomerio 3,4,3',4'-*di-cis*-, 3,4-*cis*-3',4'-*trans*-, and 3,4,3',4'-*di-trans* glycolic isomers of crustaxanthin in the ovary using the HPLC-system 1 (Fig. 1, Table 2). The observed ratio between the 3,4,3',4'-*di-cis*-, 3,4-*cis*-3',4'-*trans*-, and 3,4,3',4'-*di-trans*-glycolic crustaxanthin isomers in the ovaries was approximately 2.6:3.1:1. This suggests a relatively strong stereoselective enzymical reduction of astaxanthin to crustaxanthin in favour of the sterically hindered 3,4(3',4')-*cis*-glycolic forms in the charr.

The reduction of one ketogroup of astaxanthin to the (4'*R*)-hydroxy group of idoxanthin is stereospecific in Atlantic salmon (Schledt et al., 1988a,b), and the same may be expected in the charr (Fig. 2). Accordingly, (3*S*,3'*S*)-astaxanthin is anticipated to be the precursor of (3*S*,4*R*,3'*S*,4'*R*)-crustaxanthin, (3*R*,3'*S*)-astaxanthin of (3*R*,4*R*,3'*S*,4'*R*)-crustaxanthin, and (3*R*,3'*R*)-

astaxanthin of (3*R*,4*R*,3'*R*,4'*R*)-crustaxanthin. These represent the 3,4,3',4'-*di-cis*-, 3,4-*trans*-3',4'-*cis*-, and 3,4,3',4'-*di-trans*-glycolic forms, respectively. The different optical isomers of astaxanthin are absorbed to a similar extent by

Table 2
Relative carotenoid compositions of ovaries of sexually immature and maturing female Arctic charr

	Immature	Maturing
Iodoxanthin, % of total carotenoids	56	57
3',4'- <i>Cis</i> , % of idoxanthin	39	42
3',4'- <i>Trans</i> , % of idoxanthin	61	38
Crustaxanthin, % of total carotenoids	18	21
<i>Cis</i> , <i>cis</i> , % of crustaxanthin	40	39
<i>Cis</i> , <i>trans</i> , % of crustaxanthin	43	46
<i>Trans</i> , <i>trans</i> , % of crustaxanthin	17	15
Unidentified, % of total carotenoids	26	22
1	2	2
2 (mixture)	16	15
3 (lutein-like)	8	5



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Table 3

Relative carotenoid compositions of the skin of sexually immature and maturing male and female Arctic charr

	Immature		Maturing	
	Male	Female	Male	Female
Carotenoids, % of total				
Esterified				
Diesters	86	87	82	86
Monooesters	9	7	13	9
Unesterified carotenoids				
Astaxanthin	1	2	1	2
3',4'-Cis glycolic idoxanthin	0.1	0.3	0.1	0.1
3',4'-Trans glycolic idoxanthin	0.1	0.2	-	0.1
Unidentified carotenoids	4	4	4	3

salmonid fishes (Bjerkeng, 1997), so the crustaxanthin isomer distribution may be expected to be governed by the dietary optical isomer composition of astaxanthin.

3.3. Skin carotenoids

Skin carotenoids did not seem to be influenced by either sex or maturity status (Table 3). The skin carotenoids comprised mainly carotenol mono- (7-13% of total carotenoids) and diesters (82-87%), and only small amounts of unesterified astaxanthin (1-2% of total carotenoids) and idoxanthin (0.1-0.5%) were detected. There were also 3-4% unidentified yellow xanthophylls, but crustaxanthins were not detected in the skin samples. HPLC-analysis following saponification revealed that astaxanthin represented ca. 85%, idoxanthin 10% and unidentified yellow xanthophylls 5% of total carotenoids, respectively. This is in accord with the results presented by Scalia et al. (1989b). The ratio of 3',4'-cis to 3',4'-trans glycolic isomers of idoxanthin was ca. 1.7:1 for all fish, which differs from the ratios in the flesh and ovaries. On-line UV-Vis-spectra of the yellow xanthophylls revealed the chromophores and spectral fine-structures expected for tunaxanthin (λ_{\max} 441 nm, %III/II = 87), lutein (λ_{\max} 445 nm, %III/II = 55) and zeaxanthin (λ_{\max} 448 nm, %III/II = 20), all of which are expected metabolites of astaxanthin in fish (Schiedt, 1989, 1998; Matsuno, 1991; Schiedt et al., 1995).

Chemical reduction of astaxanthin with LiAlH₄ has been employed to determine its absolute configuration (Andrews et al., 1974), but because the skin carotenoids of Arctic charr comprise a

certain proportion of idoxanthin and its esters this analysis would yield erroneous results if employed for reduction of skin mono- and diesters of carotenols of Arctic charr (cf. Scalia et al., 1989a). The reason for this is that the idoxanthin esters present will form similar endproducts to astaxanthin, i.e. crustaxanthins.

3.4. Implications for comparative carotenoid metabolism

The transformation of dietary astaxanthin to idoxanthin appears to be a common aspect of carotenoid metabolism among fish species, occurring both in the ovary (Miki et al., 1982, 1983, 1984; Matsuno et al., 1985; Schiedt et al., 1988a,b), and in the integuments of several fish species (Schiedt, 1998), whereas crustaxanthin seems to have a more limited distribution. The occurrence of crustaxanthin in the ovaries of Arctic charr is in accord with findings for other *Salvelinus* species (Ando et al., 1990a,b). Crustaxanthin has also been detected in the muscle and ovaries of brown trout (*Salmo trutta*) and Japanese hucho (*Hucho perryi*) but was not detected in the ovaries of Atlantic salmon and rainbow trout fed diets containing astaxanthin (Schiedt et al., 1988a; Ando et al., 1990a; Bjerkeng et al., 1990).

It is not known to what extent differences in carotenoid distribution may be due to differences in affinity of specific transport proteins, although Ando and Hatano (1991) presented data indicating that the carotenoids were mainly associated with chylomicra particles in oocytes of immature salmonids, whereas they were bound to

lipovitellin and chylomiera during vitellogenesis. However, a lack of difference in carotenoid distributions between the ovarian tissues of sexually mature and immature Arctic charr in our experiment implies that the transport proteins may have a low selectivity for carotenoids.

* The salmonids appear to differ from other fish species with respect to astaxanthin metabolism. For example, farmed Atlantic cod (*Gadus morhua*) fed diets containing astaxanthin did not accumulate idoxanthin or crustaxanthin in the eggs, although possible reductive metabolites of astaxanthin were detected (Grung et al., 1993). Ovarian tissue of Alaska pollack (*Theragra chalcogramma*) contains astaxanthin esters (diesters 20-60%, monoesters 1-34% of total carotenoids) which seem to disappear as maturation proceeds, and the metabolism of astaxanthin to idoxanthin ceases (Miki et al., 1983). Crustaxanthin present in the eggs of mackerel (*Pseudopleuronectes japonicus*), yellowtail (*Seriola quinqueradiata*), and flying fish (*Prognichthys agoo*) was reported to have a mixed stereochemistry at position 4 and 4', even though the idoxanthin present had the (3S,3'S,4'R)-configuration (the *cis* glycolic form) (Matsuno et al., 1985). The authors did not, however, provide sufficient evidence to enable firm conclusions about the configuration of crustaxanthin to be drawn. The main crustaxanthin isomer extracted from the integument of gudgeon (*Pseudogobio esocinus*) was recently assigned the di-*cis* glycolic (3S,4R,3'S,4'R)-configuration (Tsushima and Matsuno, 1999). Radioactive crustaxanthin was not transformed into any detectable metabolites in the carp (*Cyprinus carpio*). Boonlawa and Olson, 1975, but this species has an oxidative carotenoid metabolism (Schedl, 1998). The role of crustaxanthin in the retinoid metabolism of fish with reductive carotenoid metabolism requires investigation.

4. Conclusion

* A reductive pathway for metabolism of astaxanthin to idoxanthin and crustaxanthin seems to be common in salmonid species, including the Arctic charr. The reduction of astaxanthin to crustaxanthin appears to be stereoselective in favour of the 3,4 (3',4')-*cis*-glycolic form, and the 3S,3'S, 3R,3'S, and 3R,3'R optical isomers of astaxanthin are anticipated to form (3S,4R,

3'S,4'R)-, (3R,4R,3'S,4'R)-, and (3R,4R,3'R,4'R)-crustaxanthin, respectively. The fish species investigated so far tend to accumulate the sterically hindered 3,4 (3',4')-*cis*-glycolic forms of crustaxanthin. The selectivity in accumulation of optical isomers of idoxanthin and crustaxanthin appears to be tissue specific, as is the case with the optical isomers of astaxanthin (Bjerkeng et al., 1997; Østerlie et al., 1999). The elucidation of the biochemical basis for the differences in carotenoid composition of different tissues will require further investigation.

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References

- Aas, G.H., Bjerkeng, B., Hatlen, B., Storebakken, T., 1997. Idoxanthin, a major carotenoid in the flesh of Arctic charr (*Salvelinus alpinus*) fed diets containing astaxanthin. *Aquaculture* 150, 135-142.
- Ando, S., Hatano, M., 1987. Metabolic pathways of carotenoids in chum salmon *Oncorhynchus keta* during spawning migration. *Comp. Biochem. Physiol.* 87B, 411-416.
- Ando, S., Hatano, M., 1991. Distribution of carotenoids in the eggs from four species of salmonids. *Comp. Biochem. Physiol.* 99B, 341-344.
- Ando, S., Osada, K., Sanezoshi, M., 1990a. Comparison of carotenoids in muscle and ovary from four genera of salmonid fishes. *Comp. Biochem. Physiol.* 93B, 503-508.
- Ando, S., Osada, K., Hatano, M., Sanezoshi, M., 1990b. Metabolism of astaxanthin in muscle and ovary from brook trout *Salvelinus fontinalis*. *Comp. Biochem. Physiol.* 96B, 355-359.
- Ando, S., Osada, K., Hatano, M., Sanezoshi, M., 1991. Characteristics of carotenoid features in muscle and ovary from anadromous and river resident types of Alaskan dolly varden charr (*Salvelinus malma malma*). *Comp. Biochem. Physiol.* 100B, 63-65.
- Andrews, A.G., Borsh, G., Llaen-Jensen, S., Sneath, G., 1974. Animal carotenoids 9. On the absolute configuration of astaxanthin and actinoerythrin. *Acta Chem. Scand.* 28B, 730-736.

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- Bjerkeng, B., 1997. Chromatographic analysis of synthesized astaxanthin — a handy tool for the ecologist and the forensic chemist? *Progr. Fish-Cult.* 59, 129-140.
- Bjerkeng, B., Storebakken, T., Liaen-Jensen, S., 1990. Response to carotenoids by rainbow trout in the sea: resorption and metabolism of dietary astaxanthin and canthaxanthin. *Aquaculture* 91, 153-162.
- Bjerkeng, B., Storebakken, T., Liaen-Jensen, S., 1992. Pigmentation of rainbow trout from start feeding to sexual maturation. *Aquaculture* 108, 333-346.
- Bjerkeng, B., Følling, M., Lagocki, S., Storebakken, T., Oli, J.J., Alsted, N., 1997. Bioavailability of all-*Z*-astaxanthin and *Z*-astaxanthin isomers in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 157, 63-82.
- Bjerkeng, B., Johnsen, K., Mayer, L., Storebakken, T., Nilssen, K.J., 1999. Influence of 11-ketotestosterone, 17 β -estradiol, and 3,5,3'-triiodo-L-thyronine on distribution and metabolism of carotenoids in Arctic charr (*Salvelinus alpinus*, L.). *Fish Physiol. Biochem.* 21, 353-364.
- Boonjawan, J., Olson, J.A., 1975. The metabolism of radioactive crustaxanthin (3,3',4,4'-tetrahydroxy- β -carotene). *Comp. Biochem. Physiol.* 50B, 363-368.
- Boyer, J.N., Van Tover, W., 1993. Reconditioning of Arctic char (*Salvelinus alpinus*) after spawning. *Aquaculture* 110, 279-284.
- Britton, G., 1995. UV/Vis spectroscopy. In: Britton, G., Liaen-Jensen, S., Pfander, H. (Eds.), *Carotenoids: Spectroscopy*, vol. 1B. Birkhäuser, Basel, pp. 13-62.
- Christiansen, J.S., Wallace, J.C., 1988. Deposition of canthaxanthin and muscle lipid in two size groups of Arctic charr, *Salvelinus alpinus* (L.). *Aquaculture* 69, 69-78.
- Crozier, G.F., 1970. Tissue carotenoids in prespawning and spawning sockeye salmon (*Oncorhynchus nerka*). *J. Fish. Res. Board Can.* 27, 973-975.
- Grung, M., Svendsen, Y.S., Liaen-Jensen, S., 1993. The carotenoids of eggs of wild and farmed cod (*Gadus morhua*). *Comp. Biochem. Physiol.* 106B, 237-242.
- Hallen, B., Aas, G.H., Jørgensen, E.H., Storebakken, T., Gøswami, U.C., 1995. Pigmentation of 1, 2 and 3 year old Arctic charr (*Salvelinus alpinus*) fed two different dietary astaxanthin concentrations. *Aquaculture* 138, 303-312.
- Hallen, B., Arnesen, A.M., Jobling, M., 1996. Muscle carotenoid concentrations in sexually maturing and immature Arctic charr, *Salvelinus alpinus* (L.). *Aquacult. Nutr.* 2, 207-212.
- Hallen, B., Arnesen, A.M., Jobling, M., Silskvuoipio, S., Bjerkeng, B., 1997. Carotenoid pigmentation in relation to feed intake, growth and social interactions in Arctic charr, *Salvelinus alpinus* (L.), from two anadromous strains. *Aquacult. Nutr.* 3, 189-199.
- Kc, B., Imsgard, F., Kjøsen, H., Liaen-Jensen, S., 1970. Electronic spectra of carotenoids at 77°K. *Biochim. Biophys. Acta* 210, 139-152.
- Kuhn, R., Sørensen, N.A., 1938. Über Astaxanthin und Ovoverdin. *Ber. Deut. Chem. Ges.* 71, 1879-1888.
- Matsuno, T., 1991. Xanthophylls as precursors of retinoids. *Pure Appl. Chem.* 63, 81-88.
- Matsuno, T., Katsuyama, M., Maoka, T., Hirano, T., Komori, T., 1985. Reductive metabolic pathways of carotenoids in fish (3S,3'S)-astaxanthin to tumaxanthin A, B and C. *Comp. Biochem. Physiol.* 80B, 779-789.
- Miki, W., Yamaguchi, K., Konosu, S., 1982. Comparison of carotenoids in the ovaries of marine fish and shellfish. *Comp. Biochem. Physiol.* 71B, 7-11.
- Miki, W., Yamaguchi, K., Konosu, S., 1983. Carotenoid composition of Alaska pollack roe at different stages of maturation. *Bull. Jap. Soc. Sci. Fish.* 49, 1615.
- Miki, W., Yamaguchi, K., Konosu, S., Watanabe, T., 1984. Metabolism of dietary carotenoids in eggs of red sea bream. *Comp. Biochem. Physiol.* 77B, 665-668.
- Østerlie, M., Bjerkeng, B., Liaen-Jensen, S., 1999. Accumulation of astaxanthin all-*E*, 9*Z* and 13*Z* geometrical isomers and 3 and 3' *RS* optical isomers in rainbow trout (*Oncorhynchus mykiss*) is selective. *J. Nutr.* 129, 391-398.
- Scalia, S., Isaksen, M., Francis, G.W., 1989a. Carotenoids of the Arctic charr, *Salvelinus alpinus* (L.). *J. Fish Biol.* 34, 969-970.
- Scalia, S., Isaksen, M., Francis, G.W., 1989b. An improved thin-layer chromatographic method for the detection of canthaxanthin in Salmonidae. *Arch. Lebensmittelhyg.* 40, 130-132.
- Schledt, K., 1989. New aspects of carotenoid metabolism in animals. In: Krinsky, N.I., Mathew-Roth, M.M., Taylor, R.F. (Eds.), *Carotenoids. Chemistry and Biology*. Plenum Press, New York, pp. 247-268.
- Schledt, K., 1998. Absorption and metabolism of carotenoids in birds, fish and crustaceans. In: Britton, G., Liaen-Jensen, S., Pfander, H. (Eds.), *Carotenoids. Biosynthesis and Metabolism*, vol. 3. Birkhäuser, Basel, pp. 285-358.
- Schledt, K., Liaen-Jensen, S., 1995. Isolation and analysis. In: Britton, G., Liaen-Jensen, S., Pfander, H. (Eds.), *Carotenoids. Isolation and Analysis*, vol. 1A. Birkhäuser, Basel, pp. 81-108.
- Schledt, K., Vecchi, M., Glinz, E., Storebakken, T., 1988a. Metabolism of carotenoids in salmonids II. Distribution and absolute configuration of idoxanthin in various organs and tissues of one Atlantic salmon (*Salmo salar*, L.) fed with astaxanthin. *Helv. Chim. Acta* 71, 881-886.

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B. Bjorkeng et al. / *Comparative Biochemistry and Physiology, Part B* 125 (2000) 395-404

- Schledt, K., Mayer, H., Vecchi, M., Glinz, E., Storebakken, T., 1988b. Metabolism of carotenoids in salmonids III. Metabolites of astaxanthin and canthaxanthin in the skin of Atlantic salmon (*Salmo salar*, L.). *Helv. Chim. Acta* 71, 887-896.
- Schledt, K., Foss, P., Storebakken, T., Liaen-Jensen, S., 1989. Metabolism of carotenoids in salmonids I. Idoxanthin, a metabolite of astaxanthin in the flesh of Atlantic salmon (*Salmo salar*, L.) under varying external conditions. *Comp. Biochem. Physiol.* 92B, 277-281.
- Schledt, K., Blohof, S., Glinz, E., 1995. Example 5: Fish. Isolation of astaxanthin and its metabolites from skin of Atlantic salmon (*Salmo salar*). In: Britton, G., Liaen-Jensen, S., Pfander, H. (Eds.), *Carotenoids. Isolation and Analysis*, vol. 1A. Birkhäuser, Basel, pp. 243-252.
- Schlep, W., Schierle, J., 1995. Example 9: Astaxanthin. In: Britton, G., Liaen-Jensen, S., Pfander, H. (Eds.), *Carotenoids. Spectroscopy*, vol. 1A. Birkhäuser, Basel, pp. 273-276.
- Shahidi, F., Synowiecki, J., Penney, R.W., 1993. Pigmentation of Arctic char (*Salvelinus alpinus*) by dietary carotenoids. *J. Aquat. Food Prod. Technol.* 2, 99-115.
- Shahidi, F., Synowiecki, J., Penney, R.W., 1994. Chemical nature of xanthophylls in flesh and skin of cultured Arctic char (*Salvelinus alpinus* L.). *Food Chem.* 51, 1-4.
- Steven, D.M., 1949. Studies on animal carotenoids II. Carotenoids in the reproductive cycle of the brown trout. *J. Exp. Biol.* 26, 293-303.
- Synowiecki, J., Shahidi, F., Penney, R.W., 1993. Nutrient composition of meat and uptake of carotenoids by Arctic char (*Salvelinus alpinus*). *J. Aquat. Food Prod. Technol.* 2, 37-58.
- Torrissen, O.J., Nævdal, G., 1988. Pigmentation of salmonids — variation in flesh carotenoids of Atlantic salmon. *Aquaculture* 68, 305-310.
- Tsushima, M., Matsuno, T., 1999. Occurrence of (3S,4R,3'R)- β,β -carotene-3,4,3'-triol and (3S,4R,3'S,4'R)- β,β -carotene-3,4,3',4'-tetrol in the gudgeon *Pseudogobio esocinus* (Cypriniformes). *Fish Sci.* 65, 500-501.
- Van, Glinz, E., Medina, V., Schledt, K., 1987. HPLC separation and determination of astacene, semiastacene, astaxanthin, and other keto-carotenoids. *J. High Resolution Chromatogr. Chromatogr. Commun.* 10, 348-351.



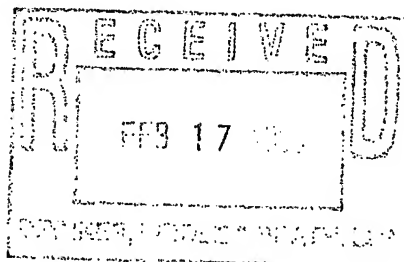
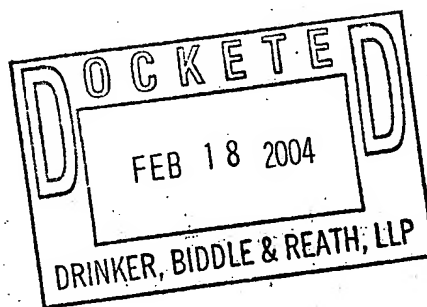
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/009,583	03/19/2002	Louise Georgina Buttle	8830-10 (157952)	1753
23973	7590	02/12/2004	EXAMINER	
DRINKER BIDDLE & REATH ONE LOGAN SQUARE 18TH AND CHERRY STREETS PHILADELPHIA, PA 19103-6996			YOUNG, MICAH PAUL	
			ART UNIT	PAPER NUMBER
			1615	

DATE MAILED: 02/12/2004

Please find below and/or attached an Office communication concerning this application or proceeding.



Advisory Action

Application No.

10/009,583

Applicant(s)

BUTTLE, LOUISE GEORGINA

Examiner

Micah-Paul Young

Art Unit

1615

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 12/24/03 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE. Therefore, further action by the applicant is required to avoid abandonment of this application. A proper reply to a final rejection under 37 CFR 1.113 may only be either: (1) a timely filed amendment which places the application in condition for allowance; (2) a timely filed Notice of Appeal (with appeal fee); or (3) a timely filed Request for Continued Examination (RCE) in compliance with 37 CFR 1.114.

PERIOD FOR REPLY [check either a) or b)]

- a) ☒ The period for reply expires 3 months from the mailing date of the final rejection.
- b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection. ONLY CHECK THIS BOX WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

1. ☐ A Notice of Appeal was filed on _____. Appellant's Brief must be filed within the period set forth in 37 CFR 1.192(a), or any extension thereof (37 CFR 1.191(d)), to avoid dismissal of the appeal.
2. ☐ The proposed amendment(s) will not be entered because:
- (a) ☐ they raise new issues that would require further consideration and/or search (see NOTE below);
 - (b) ☐ they raise the issue of new matter (see Note below);
 - (c) ☐ they are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
 - (d) ☐ they present additional claims without canceling a corresponding number of finally rejected claims.

NOTE: _____

3. ☐ Applicant's reply has overcome the following rejection(s): _____
4. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
5. ☐ The a) ☐ affidavit, b) ☐ exhibit, or c) ☐ request for reconsideration has been considered but does NOT place the application in condition for allowance because: _____
6. ☐ The affidavit or exhibit will NOT be considered because it is not directed SOLELY to issues which were newly raised by the Examiner in the final rejection.
7. ☒ For purposes of Appeal, the proposed amendment(s) a) ☐ will not be entered or b) ☒ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.

The status of the claim(s) is (or will be) as follows:

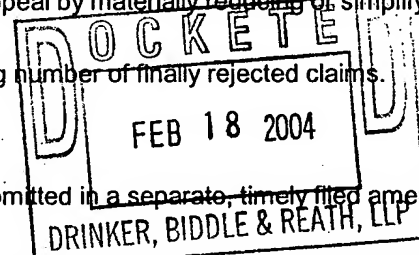
Claim(s) allowed: _____

Claim(s) objected to: _____

Claim(s) rejected: 1, 3, 4 and 12

Claim(s) withdrawn from consideration: _____

8. ☐ The drawing correction filed on _____ is a) ☐ approved or b) ☐ disapproved by the Examiner.
9. ☐ Note the attached Information Disclosure Statement(s) (PTO-1449) Paper No(s). _____
10. ☒ Other: See Continuation Sheet



Micah-Paul Young
Examiner
Art Unit: 1615

Continuation of 10. Other: The examiner agrees with applicant that salmonid and carp fish have different processes for metabolizing carotenoids. The claims however are drawn to pigments in general. Applicant has not shown that all pigments are processed differently between the two species..

LS Kishore
Goilamudi S. Kishore, PhD
Primary Examiner
Group 1500

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

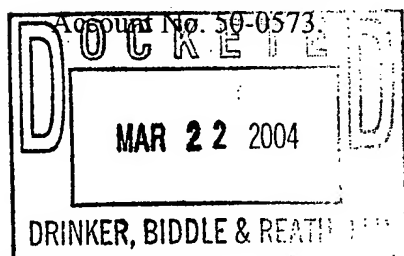
In re: Patent application of : Group Art Unit:
:poise Georgina Buttle : 1615
Serial No.: 10/009,583 : Examiner:
: Micah Paul Young
Filed: March 19, 2002 : Attorney Docket No.:
For: PIGMENT : 8830-10 (157952)

PETITION FOR EXTENSION OF TIME

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-150

Sir:


Applicants respectfully petition for a three-month extension of time to file a response to the Office Action mailed September 24, 2003. Please charge Deposit Account No. 50-0573 for the three-month extension fee of \$475.00. This extension resets the deadline for filing a response to March 24, 2004. If any additional fee is found to be due in connection with this petition, authorization is hereby provided to charge the fee, or credit any over-payment, to Deposit



Respectfully submitted,

LOUISE GEORGINA BUTTLE

BY

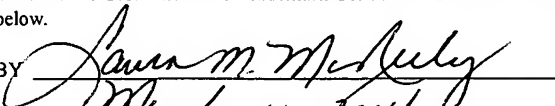

ROBERT E. CANNUSCIO
Registration No. 36,469
Drinker Biddle & Reath LLP
One Logan Square, 18th and Cherry Sts.
Philadelphia, PA 19103-6996
Tel: 215-988-3303
Fax: 215-988-2757
Attorney for Applicants

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I hereby certify that this correspondence, along with any paper referred to as being attached or enclosed, is being facsimile transmitted to the U.S. Patent and Trademark Office on the date indicated below.

BY

DATE:


March 11, 2004

Appln. No.: 10/009,583
Reply to Office Action dated Sept. 24, 2003

Docket No. 8830-10 (157952)

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Patent application of	:	
	Louise Georgina Buttle	:	
Serial No.:	10/009,583	:	Group Art Unit:
		:	1615
Filed:	March 19, 2002	:	Examiner:
		:	Micah Paul Young
For:	PIGMENT	:	
		:	Conf. No: 1753

Response Under 37 C.F.R. 1.116

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**RESPONSE UNDER 37 CFR 1.116
- EXPEDITED PROCEDURE -
EXAMINING GROUP ART UNIT**

Sir:

This is in response to the office action mailed September 24, 2003 and the Advisory Action mailed February 12, 2004. Per the petition and fee submitted herewith, Applicants request a three-month extension of time for responding to the pending office action, extending the response deadline to March 24, 2004. The petition for extension of time authorizes the Commissioner to charge the \$475 extension fee to deposit account 50-0573. If a further fee is due, kindly charge deposit account 50-0573. The Commissioner is authorized to charge any further fees due, or credit any excess, to the same account.

Amendments to the Claims begin on pg. 2 of this paper

Remarks begin on pg. 3 of this paper.

**CERTIFICATE OF FACSIMILE
TRANSMISSION**

I hereby certify that this correspondence, along with any paper referred to as being attached or enclosed, is being facsimile transmitted to the U.S. Patent and Trademark Office on the date indicated below.

BY

DATE:

Shawn M. McRuly
March 11, 2004

Amendments to the Claims:

The following listing of claims replaces all previous claim listings.

1. (Currently Amended) A method of enhancing the uptake of carotenoid pigment by fish of a salmonid species to induce a change in the pigmentation of the flesh, said method comprising the step of feeding fish with a feed having a total pellet weight, wherein the feed comprises pigment and cholesterol, and wherein the cholesterol is added to the range of 0.1-5% of the total pellet weight.

2. (Previously Cancelled)

3. (Previously Amended) A method as claimed in claim 1 wherein cholesterol comprises between 1-4% of the total pellet weight.

4. (Previously Amended) A method as claimed in claim 1 wherein cholesterol comprises between 1-3% of the total pellet weight.

5 -11. (Previously Cancelled)

12. (Previously Amended) The method of claim 1, wherein the salmonid species is Atlantic salmon, Coho salmon, Chinook salmon, Rainbow trout, or Arctic charr.

13-14 (Previously Cancelled)

Remarks/Argument

Claims 1, 3, 4 and 12 are pending in the application. Claim 1 has been amended.

Applicant's representative thanks Examiner Young for the telephone interview conducted on March 2, 2004, in which Examiner Young indicated that the pending claims would be allowable if claim 1 was amended to specify that the uptake of *carotenoid* pigment is enhanced. As discussed below, claim 1 has been amended in this manner.

This amendment places the claims in better form for allowance. Entry of the present response is therefore proper, and reconsideration of the claims is respectfully requested based on the above changes and the remarks set forth below.

PTO 1449 Forms

Applicant acknowledges that the Examiner has initialed and returned the PTO 1449 form submitted with the IDS on November 6, 2001.

Applicant respectfully requests that the Examiner initial the PTO Form 1449 submitted with the Supplemental IDS filed on March 12, 2002, and return a copy to Applicant's undersigned representative.

Response to the section 103(a) rejection

The Examiner has maintained the 35 U.S.C. 103(a) obviousness rejection of claims 1, 3, 4 and 12 over Iwahashi et al., Bulletin of the Japanese Society of Scientific Fisheries, Vol. 42, No. 12, Pages 1339-1344 (1976) ("Iwahashi"). Iwahashi discloses that skin pigmentation in decorative *carp* can be improved by the addition of carotenoids into the diet of the fish. According to the Examiner, the Applicant has established that salmonid species and carp have different processes for metabolizing carotenoids. However, the Examiner believes that the Applicants have not established that all pigments are processed differently between salmonids and carp.

Without acquiescing to the propriety of the Examiner's reasons for maintaining the obviousness rejection, and in an earnest attempt to advance prosecution, claim 1 has been amended to recite a method of enhancing the uptake of *carotenoid* pigment in salmonid fish, by feeding the salmonid fish with feed comprising pigment and cholesterol. Claim 1 as

Appln. No.: 10/009,583
Reply to Office Action dated Sept. 24, 2003

Docket No. 8830-10 (157952)

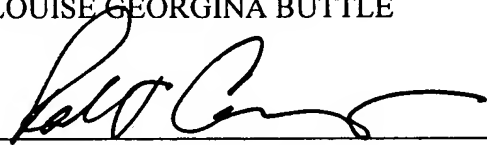
amended, and dependent claims 3, 4 and 12 are therefore non-obvious over Iwahashi. Applicant therefore respectfully requests that the 35 U.S.C. 103(a) rejection of claims 1, 3, 4 and 12 be withdrawn.

Conclusion

The claims of the application are believed to be in condition for allowance. An early action toward that end is earnestly solicited.

Respectfully submitted,

LOUISE GEORGINA BUTTLE



ROBERT E. CANNUSCIO

Registration No. 36,469

DRINKER BIDDLE & REATH, LLP.

One Logan Square

18th and Cherry Streets

Philadelphia, PA 19103-6996

(215) 988-3312 - Telephone

(215) 988-2757 - Fax

Attorney for Applicant

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File # 8830-10 (157952)

Serial/Patent/Registration # 10/009,583

Matter: PIGMENT

- ☐ Application w/Drawing
- ☐ Specimens ()
- ☐ Trans. Ltr. w/ Copies
- ☒ Response to Final O.A.
- ☐ Communication
- ☐ A.A.U./S.O.U./E.O.T.S.O.U.
- ☐ Decl. Under Sec. _____
- ☐ Renewal Application
- ☐ Ext. Time Oppose/Not. Opp.
- ☐ Specification (pgs.)
- ☐ Claims (pgs.)

- ☐ Abstract (pgs.)
- ☐ Drawings (sheets)
- ☐ Decl. and P.O.A.
- ☐ Priority Document
- ☐ Amendment
- ☐ Assignment/Merger/Chg. Nam
- ☐ Other _____

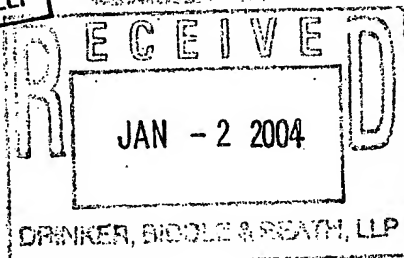
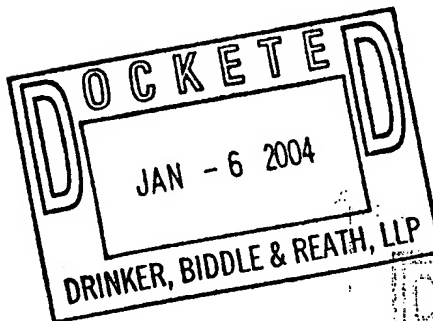
☐ Final Fee

☐ Fee \$ n/a

(Dep. Act. 50-0573)



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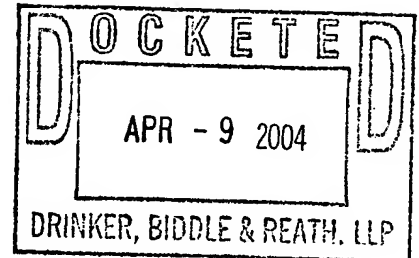
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/009,583	03/19/2002	Louise Georgina Buttle	8830-10 (157952)	1753
23973	7590	04/05/2004		
DRINKER BIDDLE & REATH ONE LOGAN SQUARE 18TH AND CHERRY STREETS PHILADELPHIA, PA 19103-6996			EXAMINER YOUNG, MICAH PAUL	
			ART UNIT	PAPER NUMBER
			1615	

DATE MAILED: 04/05/2004

Please find below and/or attached an Office communication concerning this application or proceeding.



Advisory Action

Application No.

10/009,583

Applicant(s)

BUTTLE, LOUISE GEORGINA

Examiner

Micah-Paul Young

Art Unit

1615

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 11 March 2004 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE. Therefore, further action by the applicant is required to avoid abandonment of this application. A proper reply to a final rejection under 37 CFR 1.113 may only be either: (1) a timely filed amendment which places the application in condition for allowance; (2) a timely filed Notice of Appeal (with appeal fee); or (3) a timely filed Request for Continued Examination (RCE) in compliance with 37 CFR 1.114.

PERIOD FOR REPLY [check either a) or b)]

- a) ☐ The period for reply expires _____ months from the mailing date of the final rejection.
- b) ☒ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection. ONLY CHECK THIS BOX WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

1. ☐ A Notice of Appeal was filed on _____. Appellant's Brief must be filed within the period set forth in 37 CFR 1.192(a), or any extension thereof (37 CFR 1.191(d)), to avoid dismissal of the appeal.
2. ☐ The proposed amendment(s) will not be entered because:
- (a) ☐ they raise new issues that would require further consideration and/or search (see NOTE below);
- (b) ☐ they raise the issue of new matter (see Note below);
- (c) ☐ they are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
- (d) ☐ they present additional claims without canceling a corresponding number of finally rejected claims.

NOTE: _____.

3. ☐ Applicant's reply has overcome the following rejection(s): _____.
4. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
5. ☐ The a) ☐ affidavit, b) ☐ exhibit, or c) ☐ request for reconsideration has been considered but does NOT place the application in condition for allowance because: _____.
6. ☐ The affidavit or exhibit will NOT be considered because it is not directed SOLELY to issues which were newly raised by the Examiner in the final rejection.
7. ☒ For purposes of Appeal, the proposed amendment(s) a) ☐ will not be entered or b) ☒ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.

The status of the claim(s) is (or will be) as follows:

Claim(s) allowed: _____.

Claim(s) objected to: _____.

Claim(s) rejected: 1,3-4, and 12.

Claim(s) withdrawn from consideration: _____.

8. ☐ The drawing correction filed on _____ is a) ☐ approved or b) ☐ disapproved by the Examiner.
9. ☐ Note the attached Information Disclosure Statement(s) (PTO-1449) Paper No(s). _____.
10. ☒ Other: See Continuation Sheet

Micah-Paul Young
Examiner
Art Unit: 1615

Continuation of 10. Other: Upon further review of the specification, there does not seem to be a patentable distinction made between those feeds containing cholesterol and those not containing chloesterol. The effect of the cholesterol is not fully explained or established in the specification of in the claims. The examples cite statistical data, yet the data points have SD overlaps which on face value bring into question the importance of the cholesterol. The importance of the cholesterol in the uptake enhancement of the pigment must be more clearly pointyed out and claimed in order for the prosceution to continue further. .

THURMAN K. PAGE
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1800